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Short communication

High-performance liquid chromatographic determination of paclitaxel in rat serum: application to a toxicokinetic study

François Coudoré[a,*], Nicolas Authier[a], Delphine Guillaume[b], Axel Béal[b], Eliane Duroux[b], Joseph Fialip[b]

[a]*Laboratoire de Toxicologie, Equipe NPPUA, Faculté de Pharmacie, 63001 Clermont-Ferrand, France*
[b]*Laboratoire de Pharmacologie, Equipe NPPUA, Faculté de Pharmacie, 63001 Clermont-Ferrand, France*

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Abstract

A simple and sensitive high-performance liquid chromatographic method is described for the determination of paclitaxel (Taxol®) at 230 nm using a Nucleosil C_{18} (5 μm) column and a methanol–water (70:30, v/v) mobile phase following a single-step extraction from serum with dichloromethane. The assay was validated against the classical criteria and was applied to a toxicokinetic study in rats after one or five, one per week) intraperitoneal administrations of 16 mg/kg Taxol®.

Keywords: Paclitaxel; Taxol®

1. Introduction

Paclitaxel (Taxol®) is presently one of the most important drugs used in cancer chemotherapy. It is effective against refractory solid tumours of breast or ovarian carcinoma acting via stabilisation of cellular microtubules [1]. Its clinical use is limited by leukopaenia and painful peripheral neuropathies. Reproducing this neuropathic toxicity in animals may lead to the development of new models of sensory peripheral neuropathy [2]. In humans, the grade of neuropathy is related to high accumulated doses but the pharmacokinetics is non-linear at these doses [3]. Thus, in animals, it becomes necessary to administer paclitaxel via a direct application to the peripheral nerves or by the intraperitoneal route in order to increase the exposure to the drug and the likelihood of producing neurological side effects. However, little is known about the pharmacokinetics in rodents [4–7], especially after intraperitoneal administration in mice [6,7] or in rats.

To quantify paclitaxel in human and animal samples, HPLC methods have been extensively used since 1987 [7–13]. This report describes a sensitive, simple, low cost HPLC method to analyse paclitaxel in serum, using an internal standard more commonly available than that used in previously published assays and a rapid single-step liquid–liquid extraction. This method has been validated and used to individually investigate the elimination kinetics of paclitaxel after a single or five repeated intraperitoneal injections in rats.

*Corresponding author.

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2. Experimental

2.1. Chemicals and reagents

Taxol® was kindly provided by Bristol-Myers-Squibb (30 mg paclitaxel in 6 ml of ethanol: CremophorEL, 50:50) and stored at 4°C during use. Stock solutions of paclitaxel (1 mg/ml) were prepared by dilution in methanol and stored at room temperature for a week. The internal standard, glafenine free base (Sigma, L'Isle d'Abeau, France) was firstly solubilised in HCl 0.01 *M* (1 mg/ml) then stock solutions were prepared by dilution in water (final concentration 10 μg/ml). Methanol and dichloromethane were obtained from Carbo Erba and were of HPLC-grade.

2.2. Equipment

The HPLC separations were carried out using a Waters HPLC (Waters Ass., Milford, MA, USA) system, equipped with a Model 781 UV–Vis absorbance detector, a Model 6000A solvent delivery system, a U6K injector and a Model 740 integrator.

2.3. Chromatographic analysis

The analytical column was an Interchrom Nucleosil C_{18}, 5 μm-particle size (150×4.6 mm) (Interchim, Montluçon, France). The mobile phase was prepared by mixing 350 ml of methanol and 150 ml of distilled water, and was degassed and filtered through a 0.22-μm Millipore filter before use. The flow-rate was 0.8 ml/min (2000–2200 p.s.i.) and the eluent was monitored at 230 nm. All separations were performed at ambient temperature.

2.4. Sample preparation

To 250 μl of serum were added 25 μl (250 ng) of the stock solution of glafenine and 2 ml of dichloromethane in 10-ml glass tubes. The tubes were vortexed for 2 min and centrifuged at 3500 rpm for 10 min. The organic phase (lower phase) was transferred to another glass tube and evaporated under a steam of nitrogen at ambient temperature. The residue was reconstituted with 100 μl of the mobile phase and 50 μl were injected onto the column.

2.5. Validation criteria

2.5.1. Analytical recovery

The recovery was determined by comparing the peak heights of extracts to those obtained on direct injection onto the column of the same amount of paclitaxel in methanol. Each measurement was made in triplicate.

2.5.2. Calibration curves

A working solution of paclitaxel (10 ng/ml) was obtained by 100-fold dilution of the stock solution in methanol. All other working standards (1.25, 2.5, 5 and 10 ng/μl) were prepared from this stock solution. Standard curves were obtained by adding 50 μl of each working standard and 50 μl of the internal standard (I.S.) solution to samples of pooled rat blank sera, then processing them according to the assay procedure. The ratios of the peak height of paclitaxel to that of I.S. were used to construct a calibration graph (0.18, 0.36, 0.71, 1.43 μg/ml) before each set of assays.

2.5.3. Intra- and inter-day variability and precision

Intra-day variability was tested by one technician analysing ten different samples of the same pooled biological specimen using the same calibration curves. Inter-day variability was tested on ten different days and each day a new calibration curve was constructed. Two different concentrations were tested (0.18 and 1.43 μg/ml). Precision was determined by assessing the coefficient of variation (CV, %) of the intra-day and inter-day ($n=10$) variations.

2.6. Animal studies

The method was used to assess parameters of the elimination phase of paclitaxel either after one ($n=8$ rats) or five (one per week) ($n=5$ rats) intraperitoneal administrations to rats.

Studies were performed in healthy Sprague-Daw-

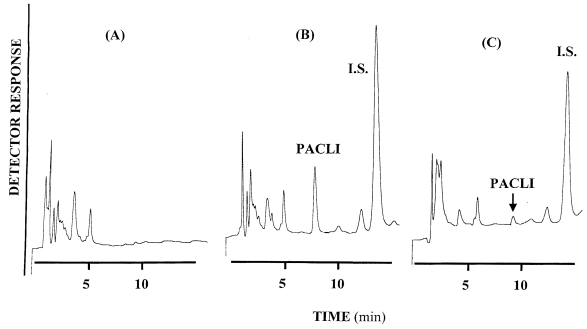

Fig. 1. Representative chromatograms of paclitaxel (Taxol®) and glafenine (I.S.) in rat extracts : (A) normal rat serum extract, (B) rat serum extract containing 0.36 μg/ml of paclitaxel, (C) rat serum extract obtained 8 h 30 min after a single i.p. injection of paclitaxel (16 mg/kg). Chromatographic conditions are described in text.

ley rats (weight range: 150–180 g) which had free access to food and water. Taxol®, dissolved in isotonic saline, was administered intraperitoneally at a dose of 16 mg/kg, at concentrations between 0.9 and 1.3 mg/ml, in order to obtain injection volumes of less than 5 ml per rat. For each rat, sampling (1–1.5 ml of blood) were performed at two different times: i.e. 8 h 30 min, 10 h, 12 h, 14 h, 16 h or 27 h after the single injection and 12 h and 14 h after the fifth injection.

Animals were anaesthetised with diethyl ether and blood samples were obtained in glass tubes from the retro-orbital sinus, blood samples were centrifuged for 10 min at 3500 *g*. Sera were frozen at −20°C until analysis. Paclitaxel levels in each rat were plotted versus time for singly or repeatedly injected rats. The half life of elimination ($t_{1/2\beta}$) was calculated using the Siphar/Win 1.2b program (Simed, Créteil, France).

3. Results and discussion

Fig. 1 illustrates representative chromatograms of a blank serum, a serum spiked with 0.36 μg/ml of Taxol® and an extract of a sample collected 8 h 30 min after a single intraperitoneal injection of 16 mg/kg of paclitaxel. No interference from endogenous substances was observed in any of the samples. Initial attempts to use acetonitrile–water as an unbuffered mobile phase, as described by Innocenti et al. [6], Eiseman et al. [7], Longnecker et al. [8], Song and Au [13] were not successful, as inconsistent retention times for Taxol and too short retention times for the first tested internal standards were obtained. Among the dozen chemicals tested as an internal standard, to avoid the use either of the costly docetaxel [11,12] or cephalomannine [7,10,13] or of substances synthesised especially for this purpose such as benzamides [5,8], we have chosen glafenine. The retention times for paclitaxel and glafenine were acceptable, i.e. 10 and 15 min, respectively.

In spite of the use of a single-step liquid–liquid extraction, without either a primary clean-up step [1] or a solid-phase extraction, the detection limit was satisfactory. It was 0.012 μg/ml based on a signal-to-noise ratio of 3, closely similar to that presented by Sparreboom et al. [1] but lower than that showed by Innocenti at al. [6] (0.125 μg/ml using 150 μl samples). The detection limit is sufficient taking into account the concentration of 43 ng/ml, at which a cytotoxicity can appear. The method was reproducible with coefficients of variation less than 12% being obtained in all cases (Tables 1 and 2). The method proved to be linear over the concentration range of 0.18 to 1.43 μg/ml of paclitaxel ($y=0.781x+0.029$, $r^2=0.9993$). The procedure for sam-

Table 1
Intra-day and inter-day accuracy for the determination of paclitaxel in rat serum

	Spiked concentration (μg/ml)	Measured concentration (μg/ml)	CV (%)
Intra-day assay	0.18	0.17±0.02	11.7
(n=10)	1.43	1.37±0.11	7.9
Inter-day assay	0.18	0.19±0.01	6.9
(n=10)	1.43	1.54±0.12	7.9

Table 2
Precision of the paclitaxel determination at different concentrations

Spiked concentration (μg/ml) (n=10)	Mean recovery (%) (mean±SD)	CV (%)
0.18	95.54±9.54	9.98
0.36	101.8±3.71	3.64
0.72	101.1±3.08	3.05
1.44	99.9±0.61	0.61

ple preparation showed a range of recovery of 90–100% similar to that observed in the literature.

Analysis of a series of unknown paclitaxel levels in rat serum from 10 to 16 h after i.p. injections yields concentrations ranging from 0.13 to 0.40 μg/ml. Comparison with data in the literature can only be done with studies in mice either after intravenous injection of 20 mg/kg [4,5] or after intraperitoneal injection of 18 mg/kg [6,7]. Our values are similar to those presented by Sparreboom et al. [4] (i.e. 0.435 μg/ml at t+8 h), but lower than those obtained by Sharma et al. [5], Innocenti et al. [6] or Eiseman et al. [7], i.e. 2.1 (8 h after i.v. injection), 3.0 (6 h after i.p. injection) and 1.0 μg/ml (12 h after i.p. injection), respectively.

In order to compare the toxicokinetic parameters of paclitaxel and especially its terminal elimination half-life time after one and five i.p. injections, rats were administered Taxol®, 16 mg/kg. This pathway of administration is particularly suited to increase intra-tumoral disposition of paclitaxel leading to higher levels in liver, colon, pancreas and ovary but also to enhance cytotoxicity. Table 3 shows that $t_{1/2\beta}$ values were higher than those presented in mice by Sparreboom et al. [1] after i.p. administration of 18–36 mg/kg (range: 2.9–3.7 h) and by Eiseman et al. [7] after i.v. injection of 22.5 mg/kg (range: 43–69 min). Such high doses of paclitaxel and possibly those of Cremophor EL, may significantly reduce drug elimination by a saturation of transport proteins involved in biliary excretion. Moreover there was no significant difference between the half-life after a single injection or after five injections, emphasising firstly the absence of accumulation of the drug between each injection, and secondly the absence of trouble in elimination pathways after five administrations of paclitaxel. Similar levels after one or five injections also suggested an unmodified transfer from the intraperitoneal cavity to blood after repeated injections.

Table 3
Terminal elimination half-life times ($t_{1/2\beta}$) of paclitaxel after single or five weekly intraperitoneal injections of 16 mg/kg to Sprague-Dawley rats[a]

Administration of Taxol®	$t_{1/2\beta}$ (min) (mean±SD)
Single injection (n=5 rats)	454.8±221.9
Five injections (n=4 rats)	470.5±94.1 (NS)

[a] Comparisons between $t_{1/2\beta}$ are made using the non-parametric Mann Whitney test (N.S.: $p>0.05$).

In conclusion, this report presents a simple isocratic reversed-phase HPLC assay for paclitaxel in serum using a rapid single-step liquid–liquid extraction technique. This method has a short analysis duration per sample with a detection limit acceptable for toxicokinetic studies.

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